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<b>(54) Title:</b> CELL DETECTION SYSTEM AND METHOD		
<b>(57) Abstract</b>  A membrane structure having controlled capillarity useful in detecting cells in a solution by promoting effective contacting between cells and the membrane surface bearing affinity for selected cell sites. Also disclosed is a method of using such membrane structures to detect particles in solution. One embodiment of the invention is an improved blood-typing device and method. The device may be attached to a blood bag, clamp, stick or the like, to provide a permanent visual record of blood type.		

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## CELL DETECTION SYSTEM AND METHOD

5     Field of the Invention

          The present invention relates to a membrane structure having controlled capillarity that allows the contacting of cells in an absorbed solution with the membrane surface. This contacting permits effective binding by cells to affinity regions on the membrane surface. More specifically, the present invention relates to a system and method of blood typing, based on immunospecific binding of blood cells to a microporous membrane and support.

15     Background of the Invention

          Affinity surface detection of cells requires the effective binding of the cells to specific binding regions on the surface. Affinity regions such as antibodies specific for antigenic regions on cells have long been known. However, the detection by the affinity surface has a serious mechanical problem, the difficulty of rapid effective binding between the affinity region and the cell, which may be in comparatively low concentration in the solution. Even more serious is the problem when the cell to be detected is fragile, such as a red blood cell (RBC). If the contacting or the conditions are such that the RBC ruptures, then detection may be impossible due to non-specific coloring. Even for other cells, the lysis of RBC in a solution often so colors the detection device so as to make the assay non-functional. Therefore, the ability to detect a cell in solution, without contacting with force sufficient to lyse cells or conditions that cause cell damage, offers a significant improvement in detection technologies. Another problem with too great a contacting force is non-specific binding. Therefore, even if the cells

fail to lyse, the binding could be non-specific if excessively strong capillarity is present.

#### Blood Cell Detection

5           One common set of detection devices is adapted to determine blood type. It is essential in supplying blood from a donor to a recipient to match the two blood types, to ensure that the recipient does not have a severe immunological response to the donor's  
10 blood. Such a response can occur when the recipient has antibodies, or can develop antibodies, to the surface antigens of the donor's blood cells.

          The most important of cell antigens, for blood-compatibility purposes, are the A and B antigens,  
15 which form the basis of the four major cell types among humans: type O (neither antigen), type A (A antigen), type B (B antigen), and AB (both antigens). The serum of the individuals of each cell type normally contains antibodies directed against the A and/or B antigens  
20 which are not present on the individuals's blood cells. That is, type O individuals have both anti-A and anti-B antibodies, type B individuals have anti-A antibodies only, and so on. To prevent the undesired immune response, it is necessary that donor blood cells  
25 not contain either A or B antigens for which the recipient has the corresponding antibodies.

          Another important antigen group which is matched is the Rh group, associated with D antigens on red blood cells. If an Rh(-) individual, whose red  
30 blood cells lack the D antigen group is given blood from an Rh(+) donor, an immune response against the foreign antigen leads to anti-D antibodies which can react with the foreign cells, particularly if a second, later transfusion of Rh(+) blood is given.

35           Antibodies for several other antigen groups, such as human leukocytic antigens (HLA), are also capable of determination using this system. This is a

crossmatching procedure for secondary blood antigen groups and it is only appropriate when individuals are receiving chronic transfusions or other continuous exposure to such secondary antigens.

5 In the usual blood-banking operation, donor blood is initially screened for A, B, and Rh type and for selected secondary antigens. Cell typing for major blood type is carried out most commonly by cell  
10 agglutination methods in which the donor blood cells are incubated with reagent anti-A and anti-B antibodies, to determine the presence of A and/or B surface antigens. Depending on the agglutination pattern, the blood can be typed as O, A, B, or AB. A similar agglutination test is used to test for Rh  
15 factor. The blood type can be confirmed by showing the ability of the donor serum, which lacks the antigen-type antibody, to agglutinate reagent red blood cells of a defined blood group.

Agglutination testing procedures commonly  
20 used in blood typing are susceptible to several types of technical and clerical errors in the testing procedure. The technician may inadvertently add the wrong reagent or blood sample to the assay mixture, or misread the agglutination result. Clerical errors can  
25 arise in transferring the blood agglutination results to the appropriate blood containers. These types of technical and clerical errors are especially difficult to avoid where hundreds of samples are being handled on a daily basis, as is common in blood banks.

30 Because of the possibility of such error, and the importance of correctly matching donor and recipient blood types, present blood donor systems include a number of backup checks to confirm major blood type. As already noted, the initial blood typing  
35 involves agglutination of donor cells, and confirmation of the blood type by serum agglutination of reagent cells of known type. Before the blood leaves the blood

bank, the major blood type groups--O, A, B, AB, and Rh--are confirmed by repeating the initial assay. The major blood groups are checked again when the donor blood is received by the hospital or clinic. The  
5 duplication needed to ensure correct donor blood type is relatively costly and time consuming both to the blood bank supplying the blood and to the receiving facility.

Cell typing methods which rely on  
10 immunospecific cell binding to a solid surface coated with cell-type specific antibodies have also been proposed. In one method, blood cells are first digested with hydrolytic enzyme, to remove surface groups which interfere with cell binding to surface-  
15 bound antibodies. The enzyme-treated cells are washed, diluted, added to microtiter wells coated with selected anti-blood group antibodies, and the microtiter plates are centrifuged to force the cells against the antibody-coated surface in each well. A positive  
20 reaction produces a monolayer of cells on the coated portion of the well surface, and a negative reaction, a discrete cell-pellet at the bottom of the well. The presence of the cell monolayer is established by reading the absorption of a monochromatic light beam at  
25 405 nm through the monolayer region of the well outside the region of the pelleted cells. (Sinor, L.T., et al., Transfusion (1985) 25:21).

Another solid-phase method is disclosed in U.S. Patent No. 4,275,053. A solid-phase support used  
30 in the method is prepared by irreversibly attaching cells having a selected cell-surface antigen type to a support matrix, then binding antigen-specific antibodies to the attached cells. In the presence of a blood sample having the selected surface antigen, the  
35 antibodies bind the suspended cells in the sample, forming a monolayer of analyte cells which are detected by standard instrumentation procedures, such as

densitometric scanning. European Patent Application 84106844 (Pub. No. 130,434) discloses the use of monoclonal antibodies in a solid lacquered film specific for blood group antigens. Other blood typing patents or publications are: USP 2,770,572; USP 4,200,690; USP 4,246,339; and USP 4,407,943; PCT W085/01354; and European Patent Application 81108009 (Pub. No. 51,748).

Many of the methods previously used for detecting blood types required the use of centrifugation to facilitate pelleting and contacting of blood cells with antibody. Such a step requires complex centrifuge equipment and further increases the possibility of errors occurring during the blood typing process. In addition, such blood typing processes are slow and require significant technical training to determine blood type.

The solid-phase blood typing methods have limited reliability, due to the weak color reaction of a monolayer of cells on a solid surface. Also, since the monolayer of cells cannot conveniently be preserved in a dehydrated form suitable for instrument analysis, the only way to confirm the original blood-group typing is to rerun the assay. The present invention solves the problems of weak color reaction, ease of use, and convenient storage by employing a dry or nearly dry membrane structure embodying a water-permeable, non-cell disruptive membrane that wicks the aqueous phase into a hydrophilic matrix thereby efficiently contacting the exposed antibodies with red blood cells.

#### Summary of the Invention

Described is a novel method of preparing a membrane structure having controlled capillarity for use in the detection of cells in a solution by effective contacting of the cells with affinity regions on the membrane structure. The membrane surface

excludes the cells from penetrating into the interior.

One application of this method provides a blood typing system which largely eliminates the possibility of technical or clerical errors, and with it, the need for multiple checks on blood type. This blood typing system comprises a dry membrane structure, which when contacted with a liquid containing blood cells, wicks the liquid into a wettable, water-permeable matrix causing firm contact between the blood cells and type specific antibody bound to the membrane. This dry membrane structure provides a membrane surface in combination with a water-permeable wicking material, that by controlled capillarity or absorbing action literally sucks or wicks aqueous phase material into the membrane thereby efficiently contacting the cells with specific antibody, without either disrupting the cells or trapping cells non-specifically.

#### Brief Description of the Drawings

Figure 1 illustrates an indicator membrane-support construct containing a solid support (10), a microporous, water-permeable, non-cell disruptive membrane surface and interior region treated to have controlled capillarity associated with the membrane surface (12), an optional spacer (14), and type specific antibody (16).

Figure 2 illustrates the indicator of Figure 1 after contact with an aqueous liquid containing red blood cells wherein the membrane-surface-interior layer (12) has absorbed liquid and red blood cells (18) are bound to specific type antibody (16).

Figures 3a and 3b illustrate a blood typing stick containing membrane regions bearing specific antibody against type A, B or D (or D<sup>u</sup>) blood antigens and the positive and negative controls.

Figure 4 illustrates a blood bag tag containing membrane-surface-interior regions bearing



specific antibody against type A, B, D (or D<sup>U</sup>) and a control region C+ which contains anti-RBC and region C- containing neutral polymer.

Figure 5 illustrates a blood typing stick containing color coded membrane regions bearing antibody against A, B, D (or D<sup>U</sup>), a control region C+ which contains anti-RBC, and a control region C- containing a non-RBC binding blocking polymer.

## 10 Detailed Description of the Invention

A dry or nearly dry membrane detector system with controlled capillarity is described, with an example of adapting the membrane system for typing blood. Methods of using such blood typing systems to determine human ABO and Rh type are illustrated.

### 15 1. Controlled Capillarity

Membrane detector devices require controlled capillarity for proper function. Absorbant porous materials are modified to produce a material having controlled capillarity through a two step process. The first step is to select a base membrane that exhibits a moderately hydrophilic character, that is, one that will draw liquid in at a moderate rate. The second step is the coating of the membrane with chemical agents that effectively control the capillary action of the base membrane materials. One example of such coating agents is polymers which make the base membrane slightly more hydrophobic, thereby controlling capillarity. While many polymers with such properties are anticipated for use as coating materials, preferred are proteins, such as serum albumin, or organic polymers, such as polyvinylchloride (PVC) and polyvinylpyrrolidone (PVP). These coating materials are dissolved in water and applied at a concentration sufficient to result in controlled capillarity of the porous absorbing material. The volume of the coating solution must be sufficient to cause even distribution

of the polymer, salt and surfactants throughout the absorbing membrane material without an excess that could dry, cake, crust, or in any other way interfere with the uniform absorption or wicking by capillary  
5 action of liquid into the porous material.

Along with the polymer, the coating solution contains salt and surfactants. The salt concentration must be at or slightly below that of isotonic saline. It may be a sodium, potassium, calcium, or other salt  
10 that will not cause the rupture of cell membranes by excessively rapid water uptake. Upon hydration of the membrane water is taken up or wicked into the membrane leaving a nearly isotonic environment within the membrane volume.

Surfactants are incorporated into the coating solution to promote uniform coating of the membrane surface and the porous interior without the detrimental buildup of salts or dissolved materials in localized regions. While any surfactant may be used, a preferred  
20 surfactant is Tween 20 (Sigma Chemical Co.) at a .01 to 1% solution, with a most preferred concentration of 0.1% vol/vol, with salt and polymer as additional ingredients.

The proper concentration of polymer in the coating solution to create controlled capillarity was  
25 determined for Bovine Serum Albumin (BSA), polyvinylpyrrolidone (PVP), and for polyvinylalcohol (PVA). Solutions of from 5 to 30% BSA (w/v) were formulated and 2 to 20 $\mu$ l of each solution applied to 6mm x 6mm  
30 membranes (Immobilon<sup>TM</sup>). The optimal BSA concentration for coating was 17% and 4 $\mu$ l was applied. For PVP solutions containing from 10 to 40% were applied to 6mm x 6mm membranes (Immobilon<sup>TM</sup>). Samples of 2 to 20 $\mu$ l were applied to each surface with optimal coating  
35 at 25% PVP and a 4 $\mu$ l volume. For PVA, solutions of from 3 to 20% were prepared and applied to 6mm x 6mm membranes (Immobilon<sup>TM</sup>). Samples applied varied from 2

to 20 $\mu$ l of solution. The optimum coating occurred at 12% PVA and 4 $\mu$ l per membrane.

Salt in the coating solution can be any non-interfering salt, but preferably sodium or potassium chloride. The concentration is isotonic or slightly below isotonic. The surfactant is applied as part of the coating solution to promote coating of the membrane surface and the porous interior of the membrane (or other porous material). Use of surfactant promotes even distribution of polymer and salt throughout the membrane surface and interior without creating buildup or crusting of the coating materials.

In use, the coating solution, containing polymer, salt and surfactant is applied after the affinity ligand (antibody, antigen, hormone, receptor, protein, etc.) has been bound to the membrane. The volume of coating solution should just fill the internal volume of the absorbing materials. In one example the membrane was 6mm x 6mm x 140 $\mu$  and the internal volume was 70% void, coating required a volume of approximately 4 $\mu$ l to produce proper distribution of the dissolved polymer, salt and surfactant. For each new membrane design, the actual quantity required can be determined by applying the solution in increasing quantities until it is no longer completely absorbed.

## 2. The Assay System for ABO Blood Test

### A. Preparing the Membrane Structure

The membrane structure in the blood assay system is composed of a substrate having surface-attached antibodies specific against a selected red blood cell surface antigen.

The substrate used in preparing the membrane structure is one which allows (a) wicking of the liquid phase into the membrane structure; (b) attachment of the antigen-specific antibodies to the substrate surface, (c) immunospecific binding of red blood cells

to the attached antibodies, and (d) modulation of the rate of water absorption by the membrane structure by varying the salt, non-specific protein concentration or charge of the membrane matrix. A variety of materials, including many polymers and glass, are suitable for the support structure of the membrane. Representative polymer materials include latex, polycarbonate, polyvinylidene fluoride (PVDF), cellulose, nylon, polyvinylacetate, polystyrene, and polyethylene. The membrane substrate material is preferably a sheet or membrane that can be cut in small patches or pads, such as 2-8mm disks, squares, etc., although other substrate forms, such as beads or rods, may be employed, as will be appreciated below. The substrate may provide, or be modified to provide, surface chemical groups, such as carboxyl, hydroxyl, aldehyde, sulfhydryl, amide, or amine groups, which can participate in covalent chemical linkages used in attaching the antibody to the support. Similarly, linkages may also be through ether bonds. One preferred substrate material used in preparing the supports described in examples I-IV is a chemically activated hydrophilic microporous membrane to which antibodies can be covalently attached such as Immobilon<sup>TM</sup> by Millipore Corporation.

The base membrane material of the Immobilon<sup>TM</sup> membrane is a non-interactive fluorocarbon polymer. However, any membrane that excludes the penetration of cells and that allows immobilization of proteins or other affinity agents while retaining native activity will be sufficient. The pore sizes of the Immobilon<sup>TM</sup> membranes range from 0.65 $\mu$ m to 5 $\mu$ m.

The hydrophilic microporous membrane must be adjusted to absorb the aqueous phase at a proper rate that is controlled by capillarity. The rate of absorption is controlled by the addition of salts such as potassium or sodium, and by the addition of non-specific polymers. Surface charge or pH may also

effect binding. If the rate of water absorption is so high it results in non-specific binding of cells or cell rupture, then the non-specific polymer is increased, thereby decreasing the rate of water  
5 absorption.

The preferred temperature for running a determination of blood groups is room temperature  $\pm 5^\circ$ . However, in the presence of blood rich in lipid components, the optimum temperature may be higher, in  
10 the range of 30-40°C or more. The elevated temperature is required due to the increased viscosity of the blood fraction caused by the lipid phase.

Among the types of affinity regions are any ligand/ligand receptor, such as antibody-antigen,  
15 hormone-receptor, enzyme-substrate, or any two molecular means having binding affinities to one another, with one means in solution and the other means attached to the membrane, thereby facilitating detectable isolation of the cells in the solution.

20 The cell-specific antibodies attached to the substrate are commonly mouse or human IgM or IgG antibodies which are specific against selected human red cell antigens, and particularly the A, B, and D cell antigens associated with A, B, and Rh(+) human  
25 blood groups. Mouse and human IgM antibodies specific against human blood group A, B groups are available commercially, either as partially purified antisera or in purified form. Anti-D antiserum or monoclonal antibodies for binding to Rh-associated antigens, can  
30 also be obtained commercially, and the anti-D antibody can be further purified by known methods. The antibodies may be either polyclonal or monoclonal.

Attachment of antibodies may be accomplished by the use of coupling agents such as glutaraldehyde,  
35 carbonyldimidazole, or trichlorotriazine by methods well-known in the art.

Bethell, G.S., Ayers, J.S., Hancock, W.S. and Hearn, M.T.W. (1979) J. Biol. Chem. 254:2572-2574.

Zuk, R.F., Ginsberg, V.K., Houts, T., Rabble, J., Merrick, H., Ullman, E.F., Fisher, M.M., Sizto, C.C., Stiso, S.N., Litman, D.J. (1985) Clin. Chem. 31:1144-1150.

In a typical procedure, the antibody protein is covalently coupled to the membrane substrate through reactive chemical groups on the substrate surface. The coupling may involve direct chemical bonding between a reactive surface group, such as an aldehyde group, and a suitable protein group, such as primary amines, or may involve cross-linking by means of a suitable bifunctional linking reagent, such as glutaraldehyde. A variety of known coupling reactions are available. Alternatively, for some substrate materials, the secondary antibodies can be adsorbed tightly to the substrate without covalent attachment. In either method, the substrate is washed thoroughly after the binding reaction to remove unbound material. After washing to remove unbound antibody material, the remaining active protein binding sites can be blocked by the addition of other proteinaceous or polymeric material. This protein or polymer material binds to those coupling sites that are not occupied by antibody, thereby reducing or eliminating the non-specific binding of proteins in the test material. One such group of proteins are the non-immunoglobulin components of serum such as alpha-2-macroglobulin and albumin. These proteins may accompany the antibody to complete the binding and blocking reactions simultaneously. Another protein useful for such blocking is the milk protein casein or any other protein that has no affinity for the antigen to be detected.

The membrane material itself may be water absorbing, such as the material Immobilon<sup>TM</sup> or it can be the membrane in combination with any water-absorbing

material such as paper or a hydrophylic polymer. One preferred method of assembling the membrane and the support element is to first bind the membrane component to the support component by the use of a double-sided adhesive, followed by the binding of the specific antibody and adjustment of the membrane to absorb water at the rate appropriate to contact the blood cells with the membrane surface antibodies at a rate that is designated as controlled capillarity. The adhesive assembly of a membrane, already bearing antibody, to the support element results in an unpredictable rate of water absorption thereby increasing the likelihood of non-specific binding of red blood cells or the lysis of red blood cells.

Figure 1 illustrates a support (10) in combination with a water permeable membrane (12) a chemical linker (14) and an antibody (16). Any bifunctional chemical or polymer may be used as a linker, or no linker can be used. Figure 2 illustrates a support (10) adjacent to a membrane (12) which is attached to a linker (14) which in turn is bound to an antibody (16) which is schematically bound to a cell such as a red blood cell. Each cell is bound by multiple antibodies to hold it to the membrane support device. The number of antibodies binding to the cell is that sufficient to retain it during the washing process, preferably 20 or more antibody molecules per cell contact surface area.

Figures 3a, 3b and 4 show a stick (20) or a tag (33) which contains membrane pad regions of the type described with reference to Figures 1 and 2. In one embodiment with dimensions of 6mm x 6mm 140 $\mu$  (70% void volume). Three of the membranes, which are indicated at A, B, and D, have surface antibodies specific against blood groups A, B, and D (Rh), respectively. The fourth and fifth pads, the positive control containing anti-RBC antibody and the negative

control having non-specific antibodies or non-antibody proteins or other polymers may optionally be present. The supports may be formed on separate membranes and then attached, as by adhesive, to a tag, or the tag  
5 itself may be the membrane on which the five pad regions are formed. The tag is preferably white or otherwise light colored to enhance color contrast with the blood cell matrix which is developed on the membrane supports.

10 Figure 3a illustrates a stick (20) with the five membrane-pad areas which are an anti-A antibody area (25), an anti-B antibody area (24), an anti-D antibody area (23), a positive control pad bearing antibody against all red blood cells (22), and a  
15 negative control (21) bearing neutral protein or protein without affinity for red blood cells. In Figure 3a all the pad areas are uncolored. In Figure 3b the pad areas 28, 29, 30 and 31 are speckled indicating the binding of red blood cells. This  
20 indicates that the blood type is AB positive and the assay has a positive control (28) and a clear negative control (27) indicating no non-specific absorption of red blood cells. Each pad can be individually adjusted for rate of water absorption to compensate for the  
25 varying affinity of the antibody for the red blood cell surface antigen. That is a higher rate of water absorption may be needed to effectively contact lower affinity antibody with cell surface antigens.

The tag is preferably designed for attachment  
30 to a blood container to provide a tag on the container identifying the blood type of the contents. In Figure 4 there is shown a portion of a blood bag (32) having a blood-type identification card, such as a tag (33) adhesively attached to a side of the bag. The  
35 particular card shown indicates type B/Rh(+) blood. The method of performing blood group determination assays on the card will be described below.



Soluble antibodies which are specific against cell surface antigens other than the blood-group antigen being tested may also serve to detect cell types according to the method of the invention. One such method is using human leukocytic antigens (HLA) specific antibodies. Methods for preparing such antibodies are well known.

### 3. Blood-Typing Assay Method

10 The method of the invention involves, first, providing a membrane support of the type described above, constructed for binding specifically and with high affinity to red blood cells having selected cell surface antigens, typically antigens characteristic of blood group A, B, or Rh factor. The membrane must be 15 dry or nearly dry when used to facilitate wicking of the aqueous phase into the membrane.

A sample of the whole blood or blood cells to be typed are added to each membrane in an amount 20 sufficient to cover the membrane, exposing the membrane to the suspended sample cells. Alternatively, a stick or tag bearing the membrane may be immersed in the sample. In a diluted sample containing comparatively few cells, a thicker membrane may be required to absorb 25 a greater quantity of solution to contact the required number of cells with the membrane surface. For example, when a sample has been diluted N times, the associated interior absorbing area must also be increased about N times.

30 Figure 3b illustrates the binding of cells to a diagnostic stick bearing a membrane which occurs when the membrane is exposed to the cells in aqueous or whole blood solution. The diagnostic stick in Figure 3b consists of a backing (20), membrane-wicking region 35 bearing anti-human blood type antibodies (22-25) which are attached to the membrane surface as in Figures 1, 2 and 3a. In Figure 3b, the bound red blood cells form a

dense cell layer (28-31) that is easily detected visually or by scanning devices.

The washed support(s) may be preserved in permanent form by drying, leaving strongly colored  
5 dried-matrix areas. If desired, the dried supports (or support regions on a card) can be protected with a spray-on lacquer or the like.

Illustrating the method of the invention as it might be practiced by a blood bank, donor blood is  
10 collected in a blood bag, such as bag (32) (Figure 4). Blood from the bag is then applied to the support regions of a tag (33). After incubation for 1-3 minutes at room temperature, the card is washed--either by rinsing the tag with a physiological saline  
15 solution, or immersing the tag in such a solution, with gentle agitation. The tag now shows strongly colored regions where immunospecific cell binding has occurred, and virtually uncolored regions for the negative regions. In the embodiment illustrated, the assay  
20 indicates type AB/Rh(+) blood. The card may be allowed to dry, giving a permanent, easily readable record of blood type. The card can be permanently attached, for example, by stapling, clamping or adhesive backing, to the blood container, in a blood donor setting. In the  
25 case of a blood recipient, the card or strip can be attached to the recipient's hospital record or directly to a patient in the form of a bracelet.

Numerous embodiments of the cell detection membrane system are anticipated. Among the mammalian  
30 cells anticipated are blood cells, including human and other mammals such as horse, cow, sheep, goat, pig, dog, cat, rabbit, rat and mouse; and eukaryotic tissue culture cell lines. When the cell is detectable due to its own inherent properties, such as red color for red  
35 blood cells, then no additional agent is required for detection. However, where the cell is not sufficiently detectable, agents such as dyes, detectably labeled

antibodies, or detectably labeled affinity ligands must be used. Among the detectable agents are radioisotopes, enzymes, fluorescent dyes, and electron opaque materials.

5           General embodiments of the cell detection devices are illustrated in Figures 1-5, with a specific detector drawn to scale in Figure 5. The cell detection support device may be any material offering support that does not interfere with detection.

10          Detectors may be in various forms including pads, sticks, tags, bracelets, cards or other embodiments suitable for easy utilization and manufacture.

          A specific embodiment, as illustrated to scale in Figure 5, is a cell detector with membrane  
15          surfaces suitable for detecting specific types of red blood cells. The diagnostic strip consists of a support strip (44) suitable for holding by hand for insertion into blood containers. The dimensions anticipated are from 2-6 inches, preferably from 3-5  
20          inches, and most preferably 4.25 inches in length. The width may be from 2-15mm, preferably 3-9mm, and most preferably 6mm. Attached to the support are membrane surfaces containing antibody affinity regions as described in Example I. In this embodiment there are  
25          the following distinct regions on the blood typing device: region (34) which is free of membrane to ensure that sediment at the bottom of the solution does not interfere with detection; region (35) containing a membrane with antibody to human D or D<sup>u</sup>; region (36)  
30          with grey lettering "Rh", or with a grey background and lettering of a different color; region (37) containing a membrane with antibody to human B antigen; region (38) with yellow lettering "B" or with a yellow background and lettering of a different color; region  
35          (39) containing a membrane with antibody to human A antigen; region (40) with blue lettering "A" or with a blue background and lettering of a different color;

region (41) an open space; region (42) and (43) are membranes for control purposes, one a positive control membrane containing antibody non-specifically binding red blood cells and the other a negative control  
5 membrane with a blocking agent such that there is no specific affinity for red blood cells. The order of the membranes on the stick may be changed, the size of the membranes and lettering may be altered, however, the colors are a critical aspect of this embodiment of  
10 the invention. This unique design of a diagnostic stick for blood typing embodies the membranes with controlled capillarity and color indicators in compliance with 21 CFR Section 660.28.

Other embodiments of this invention will have  
15 indicator regions with a pink "C", a brown "E", an orange "CDE", a lavender "c", or a green "e"; or a similar color background with a different color for the letter.

From the foregoing, it can be appreciated how  
20 various objects and features of the invention are met. The blood typing system substantially eliminates the possibility of technical errors due to mix-ups in blood samples or reagents. In the embodiment of the invention illustrated in Figure 4, blood samples are  
25 transferred directly from a blood bag or sample to a card, and the only reagent which is added to the card is a saline wash. Clerical errors are minimized since a visual record of blood type is contained on the card itself.

30 Unlike other blood typing methods proposed heretofore, the binding reaction in the present invention is easily read visually, i.e., without special spectrophotometer or densitometer equipment and without a requirement for complex interpretation or  
35 analysis.

Another important advantage of the invention is that the original assay can provide a permanent

record of the assay results, so retesting need not be required to confirm blood type.

The following examples are not to be limiting but rather illustrate specific embodiments of the invention.

## EXPERIMENTAL

### Example I

#### 10 Preparation of Membrane

##### With Anti-A, Anti-B, or Anti-D Antibody

To a sheet of support or backing material are applied a series of strips of membrane material by the use of double-sided adhesives (Immobilon<sup>TM</sup>, Millipore Corporation; Adhesives, 3M Corporation). Antibodies  
15 against the A, B or D human red blood cell antigens are commercially available (Gamma Biologics, Inc., Houston, TX). These antibodies may be either monoclonal or polyclonal in nature. Following application of the  
20 membrane to the support surface there are five steps which are: binding, drying, coating, drying and storing.

In the first step the antibody plus optional inert proteins or polymers such as  $\alpha$ 2-macroglobulin in  
25 phosphate-buffered saline is applied to the membrane. A volume of approximately 10 $\mu$ l of this liquid containing antibody is applied per 6mm square area of membrane. The antibody solution contains from 0.05 $\mu$ g to 0.5 $\mu$ g of antibody per 10 $\mu$ l. The concentration of  
30 polymer or protein, when present, is approximately 100 $\mu$ g per 10 $\mu$ l. Prior to binding of the antibody the membrane surface had been treated with the necessary coupling reagents. Following the binding of antibody to the membrane there is a 20min drying period at 27°  
35 or for a longer period of time until dry. Once the drying period is completed the next step is coating of the membrane to create the controlled capillarity. To

control capillarity a coating solution containing a salt, a surfactant, and a polymer such as a protein or other polymer was applied. One such coating solution containing 20% BSA, the surfactant Tween 20, and sodium chloride in deionized water was used. A 5 $\mu$ l sample of this coating solution was applied for each 6mm square area of the membrane.

After the coating step the coated membrane was dried for 20min at 37° or until dry.

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### Example II

#### Preparation of Diagnostic Sticks

The sheet bearing the treated membrane was then cut into strips 6mm wide. These strips could then be stored in a dry, cool or frozen condition until needed. Each membrane area on the strip contained membrane areas 6mm x 6mm x 140 $\mu$  with an internal volume of 3.5 to 5 $\mu$ l.

The support sheet bearing the membrane treated with antibody solutions against human antigens A, B and D were cut into strips 6mm or less in width and 4.25 inches or greater in length. These cut strips were then stored in a dry container containing a desiccant. They may be kept in either a cool or a frozen condition. When needed, the diagnostic strips are removed for use.

### Example III

#### Preparation of Diagnostic Tags

Diagnostic tags are prepared by the method similar to that of Example I with the antibody solution and the coating solutions applied to membrane disks attached to a support backing. The support backing has on its opposite side an adhesive backing suitable for application to a container, bracelet or document.

Example IVTest for A, B, AB, O or D Blood Type

Whole blood from A, AB, and O-group donors was obtained from a local blood bank.

5 Individual membrane diagnostic sticks having membrane surface-bound mouse anti-human blood group A, B, D and RBC prepared as in Example I and Example II were each placed into test tubes bearing whole blood for 60 seconds. The diagnostic stick was then removed  
10 and washed with a saline solution (approximately 5-10 ml) or immersed into a saline filled test tube (30-50 ml) for 10-30 seconds. The diagnostic stick was then read.

Visual inspection of the membranes showed:

15 (a) The A and AB blood types gave a strong color reaction (membrane surface coatings) on disks containing surface-bound anti-A antibodies;

(b) The B and AB blood types gave a strong color reaction on disks containing surface-bound anti-B  
20 antibodies.

(c) No appreciable blood cell binding of O-group blood to either anti-A or anti-B membrane was detected.

25 Table 1 summarizes the results for tests with A+, A-, B+, B-, AB+, AB-, O+ and O- human blood. The red indicating binding. Figures 3b and 4 indicate the pattern for AB+.

TABLE 1

	Blood Types							
	A+	A-	B+	B-	AB+	AB-	O+	O-
Anti-A	+	+	-	-	+	+	-	-
Anti-B	-	-	+	+	+	+	-	-
Anti-D	+	-	+	-	+	-	+	-
Anti-RBC (C+)	+	+	+	+	+	+	+	+
35 Neg-Cont (C-)	-	-	-	-	-	-	-	-

Key: + = Red; - = White

Therefore, the results of Table 1 indicate the detection of mammalian red blood cell type using membrane device having controlled capillarity.

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WHAT IS CLAIMED IS:

1. A structure having controlled capillarity for use in contacting cells in solution with a membrane comprising:
  - 5 a) a porous membrane surface;
  - b) a porous interior capable of incorporating said solution at a controlled rate sufficient to effectively contact said cells with said membrane surface.
- 10 2. The structure of Claim 1 wherein said membrane surface and said porous interior are composed of different materials.
- 15 3. The structure of Claim 1 wherein said membrane surface and said porous interior are composed of the same material.
- 20 4. The structure of Claim 3 wherein said membrane is attached to a support means capable of maintaining the membrane topology during manipulation.
- 25 5. The structure of Claim 4 wherein said cells are one of the following: red blood cells, white blood cells, tissue culture cells, or whole blood.
6. The structure of Claim 5 wherein said cells are the human red blood cells.
- 30 7. The structure of Claim 6 for use in typing red blood cells, comprising:
  - said membrane surface having a non-cell disruptive membrane surface, having pore sizes less than about 5 microns;
  - 35 said interior membrane having controlled capillarity for drawing a liquid sample placed in contact with said membrane surface through said

membrane surface; and

attached to said membrane surface, a surface array of type-specific antibodies capable of binding red blood cells in a selected-type blood sample immunospecifically, when the sample is placed against the membrane and the red cells are drawn against the membrane.

8. The structure of Claim 7, wherein the liquid volume capacity of said interior is sufficient to draw against the membrane, a volume of blood sample which contains at least that number of red blood cells necessary to form a visible layer of immunospecifically bound red blood cells on said membrane surface.

9. The structure of Claim 8, for determining blood type in a blood sample which has been diluted N times with an aqueous assay medium, wherein the membrane and associated interior are formed as a unitary structure having a thickness of at least about N times 20 microns.

10. The structure of Claim 8, wherein said membrane is composed of polymer fibers composed of one of the following: PVDF, PTFE, modified nylon nitrocellulose, regenerated cellulose, or cellulose.

11. The structure of Claim 10, wherein said membrane surface is substantially uncharged.

12. The structure of Claim 7, wherein the antibodies are attached by adsorption to said membrane surface.

13. The structure of Claim 7, wherein said antibodies are attached covalently to said membrane surface.

14. The structure of Claim 13, wherein said membrane surface and interior are formed as a unitary PVDF fiber membrane, and said antibodies are attached to said membrane surface by carbodimidazole coupling.

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15. The structure of Claim 7, wherein said antibodies are selected from the group consisting of anti-A, anti-B, and anti-D antibodies.

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16. The structure of Claim 15, wherein said antibodies are immunoglobulin M anti-human D monoclonal antibodies, and the antibodies on said membrane surface are effective in binding both D and D<sup>u</sup> type human red blood cells at a surface concentration sufficient to  
15 form a visually identifiable cell monolayer on the membrane surface.

17. A blood typing device for determining A, B, AB, O, RhD, and RhD<sup>u</sup> blood type comprising:  
20 first, second, and third membrane structures;  
each membrane structure being composed of a wetable, water-permeable, non-cell disruptive, membrane surface having pore sizes less than about 5 microns, and an interior region operatively associated with said  
25 membrane surface for drawing by controlled capillarity a liquid sample placed against said membrane surface through said membrane surface;

said first structure having attached to its membrane a surface array of anti-A blood type antibody  
30 capable of binding red blood cells immunospecifically when a type A or type AB blood sample is placed against the membrane, and the cells are drawn against the membrane by said controlled capillary action of the interior of said membrane structure;

35 said second structure having attached to its membrane a surface array of anti-B blood type antibody capable of binding red blood cells immunospecifically

when a type B or type AB blood sample is placed against the membrane, and the cells are drawn against the membrane by said controlled capillary action of the interior of said membrane structure; and

5           said third structure having attached to its membrane a surface array of human anti-D blood type antibody capable of binding red blood cells immunospecifically when a type D or type D<sup>u</sup> blood sample is placed against the membrane, and the cells  
10 are drawn against the membrane by the controlled capillary action of the interior of said membrane structure.

18. The device of Claim 17, wherein the anti-  
15 D antibody attached to the third structure is an immunoglobulin M human monoclonal antibody.

19. The device of Claim 17, which further includes a fourth membrane structure and having  
20 attached to its membrane surface an array of anti-human red blood cell antibody capable of binding red blood cells immunospecifically when a blood sample is placed against the membrane, and the cells are drawn against the membrane by the controlled capillary action of the  
25 interior of said membrane structure, and a fifth membrane structure having attached to its membrane surface an array of polymer which have no immunospecific binding affinity for red blood cells.

30           20. The device of Claim 17, wherein the antibodies are attached to one of the following selected membranes as follows:

(a) covalently, by means of cyanogen bromide, to a PVDF filter; and

35           (b) by adsorption to PTFE, nitrocellulose, modified nylon, regenerated cellulose or a cellulose filter.

21. A method of typing red blood cells in a blood sample comprising:

providing a membrane structure composed of a wetable, water-permeable, non-cell disruptive, membrane surface having pore sizes less than about 5 microns, an interior region operatively associated with the membrane surface for drawing by controlled capillarity a liquid sample placed against the membrane surface through said membrane surface, and, attached to said membrane surface, a surface array of type-specific antibodies capable of binding red blood cells immunospecifically in a selected-type blood sample; and placing a sample in contact with the membrane; and

by said placing, causing red cells in the sample to be drawn against the membrane by action of the interior controlled capillarity, whereby type-specific cells react immunospecifically with such antibodies to form a monolayer of red blood cell on the membrane; and

washing said membrane to remove red blood cells adhering nonspecifically to the membrane; and detecting the presence of said layer of red blood cells as an indication of blood type.

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22. The method of Claim 21, for use in determining A, B, AB, O, RhD, and RhD<sup>u</sup> blood types, wherein three such membrane structures are provided, the first having anti-A antibodies specific against A and AB type blood, the second having anti-B antibodies specific against B and AB type blood, and the third having human anti-D antibody specific against both D and D<sup>u</sup> type blood.

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23. The device of Claim 17, wherein the horizontal dimension is 6mm or less and the vertical dimension 4.25 inches or greater.

24. The method of Claim 7, wherein the pore sizes are less than 2 microns.

25. The method of Claim 24, wherein the pore size is about 0.65 microns.

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26. The device of Claim 23, wherein the said device is a blood typing stick substantially as illustrated and described in Figure 5.

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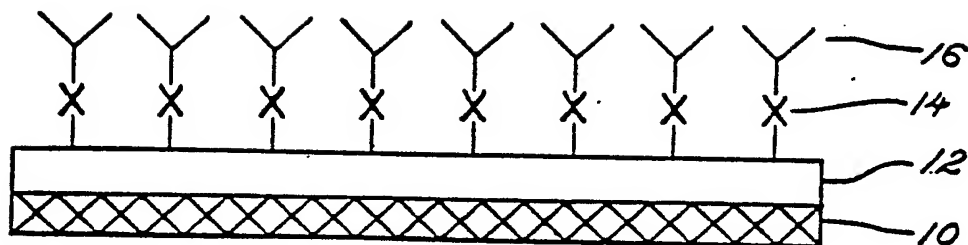


FIG. 1

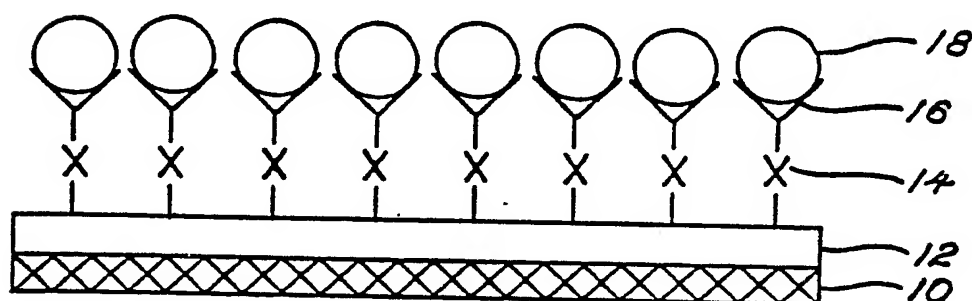


FIG. 2

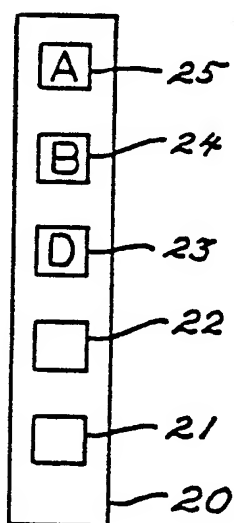


FIG. 3a

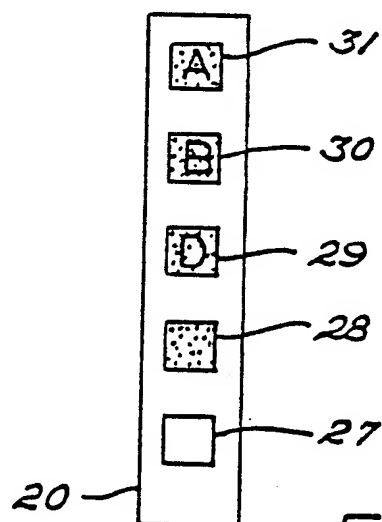


FIG. 3b

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FIG. 4

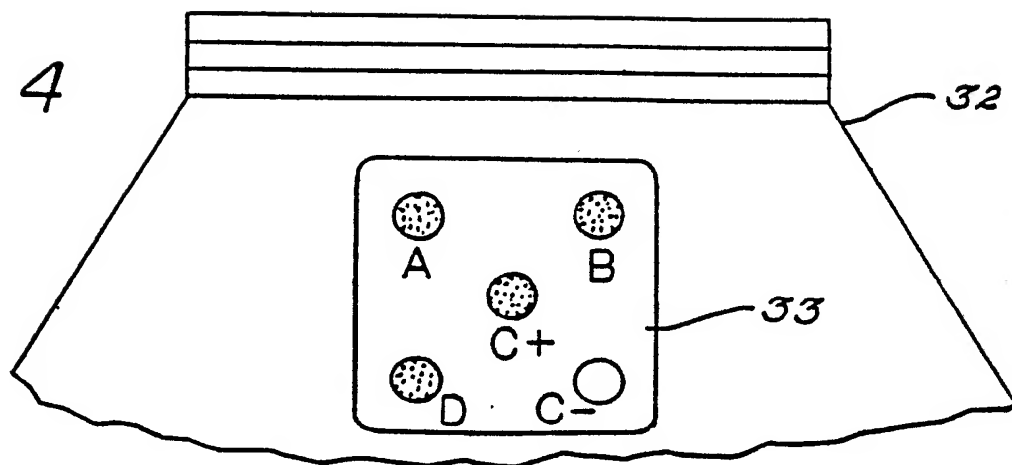
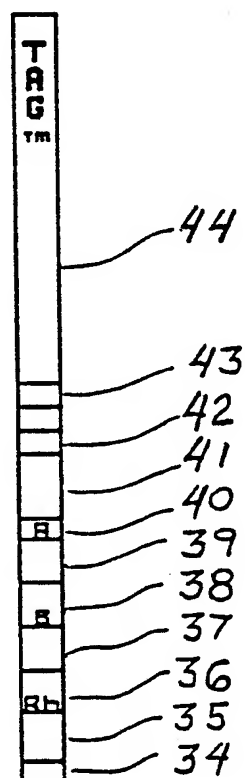


FIG. 5





# INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00838

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>3</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): C12Q 1/00; G01N 33/53; G01N 33/543 U.S. CL: 435/4, 7, 436/513, 518		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
U.S.	435/4, 7, 29 436/63, 513, 518, 519, 520, 528, 529, 530, 531, 535, 807	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>5</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>		
Category *	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
X Y	US,A, 4,033,817 (GREGOR) 05 July 1977, see entire document.	1-4 <hr style="width: 50%; margin: 5px auto;"/> 5-26
P,Y	US,A, 4,594,327 (ZUK) 10 June 1986, see entire document.	1-26
Y	US,A, 4,275,053 (ROSENFELD ET AL) 23 June 1981, see entire document.	1-26
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>2</sup>		Date of Mailing of this International Search Report <sup>2</sup>
14 July 1987		31 JUL 1987
International Searching Authority <sup>1</sup>		Signature of Authorized Officer <sup>20</sup>
ISA/US		 Stephen C. Wieder